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QUANTITATIVE ORGANIC SEMI-MICRO METHODS OF COMBUSTION.*

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The earliest attempt at the development of a semi-micro method of determining the percentage of carbon and hydrogen in organic compounds was that of Collie (*J. Chem. Soc.*, 1904, **85**, 1111). He devised an apparatus by which the estimations were carried out by a purely gaso-volumetric procedure using 15 to 20 mg. of substances. Similar methods were put forward later by Pouget and Chouchack (*Bull. Soc. chim.*, 1908, **3**, [4], 75), Hackspill and Heeckeren (*Compt. rend.*, 1923, **177**, 59) and Hackspill and D'Huart (*Ann. Chim.*, 1926, **5**, [10], 96).

Wise (*J. Amer. Chem. Soc.*, 1917, **39**, 2055) was the first to adapt the micro methods of Pregl to centigramme analysis. On account of the relatively larger quantities of substances taken, he carried out the combustions in a stream of oxygen, using platinised asbestos as catalyst and asbestos impregnated with copper oxide as the oxidising material. The products formed were collected in absorption vessels similar to those of Pregl. Lauer and Dobrovolny (*Mikrochemie*, Pregl-festschrift, 1929, p. 243) closely follow Wise in the manner of filling the combustion tube and introduce, further, some of the special features of Pregl's method, such as the pressure regulator, etc. Reference must also be made to the contributions of Polonowski (*Bull. Soc. chim.*, 1924, **35**, [4], 414) and Wahl and Sisely (*Compt. rend.*, 1928, **186**, 1555; *Bull.*

* This paper is taken from the thesis submitted by S. Siddappa for the degree of Master of Science, in the University of Mysore.

Soc. chim., 1928, **43**, [4], 1279). The latter recognized the advantages resulting from the taking of small amounts of material for combustions and described a procedure which was an adaptation of the classical method of Liebig.

Heslinga (*Rec. trav. chim.*, 1924, **43**, 551) introduced specially prepared manganese dioxide as a catalytic oxidising agent in semi-micro analysis. This substance, however, has to be frequently regenerated outside the combustion tube by a wet process. In the method of Berl and Burckhardt (*Ber.*, 1926, **59**, 890) lead chromate is employed as the oxidising material. The water formed is absorbed by conc. sulphuric acid, and carbon dioxide is estimated either gaso-volumetrically or by absorption in a soda-lime tube. Later, for the analysis of nitrogenous organic compounds lead peroxide was introduced into the combustion tube, according to the method of Pregl (Berl, Schmidt and Winnacker, *Ber.*, 1928, **61**, 83). But the principal disadvantage in the method of Berl and collaborators is that the weighed material has to be mixed with dry powdered lead chromate and introduced into the combustion tube as in the case of nitrogen determinations.

Sucharda and Bobranski's publication "Halbmikromethoden zur automatischen Verbrennung organischer Substanzen und ebullioskopischen Molekulargewichtsbestimmung" appeared in 1929. Their methods follow those of Pregl very closely even in the matter of small details, and by means of a very ingenious device they make the process of combustion completely automatic. When we attempted to work this method in our laboratory we found that the automatic regulator would not function* possibly owing to differences in the nature of the gas supply and its pressure. Further experiments have led us to several modifications and improvements which simplify the process considerably. The result has been the evolution of methods which may be claimed to be of general applicability.

In developing the present methods the following points have been kept in view:

(*a*) to simplify the apparatus and procedure so that they may replace the macro methods in regular instruction,† and

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* *Cf.* Calvet and Mosquera, *Chem. Abs.*, 1933, **27**, 244.

† Berger, *J. pr. Chem.*, 1932, **133**, [ii], 1. See also Hans Meyer, "Analyse und Konstitutionsermittlung Organischer Verbindungen," 5th ed., 1931, pp. 99-109 and p. 124; Weygand, "Quantitative analytische Mikromethoden der organischen Chemie," 1931, pp. 130-148 and 54-66.

(*b*) to follow closely the micro methods of Pregl so that the experience gained here may serve as a valuable preliminary to training in organic quantitative micro analysis.

The principal advantages are:

(*a*) very considerable saving in materials,

(*b*) comfort and convenience in working, and

(*c*) shortness of the duration of the analyses.

It is hoped that as a result of the publication of this paper semi-micro methods of combustion will come into more general use.

THE BALANCE.

Although a micro balance is not necessary, an accurate balance by which the fourth place after the decimal can be determined with absolute certainty is essential.* It will, however, be a great advantage to have a balance wherein the fifth place after the decimal can be satisfactorily estimated. The pans should be fitted with side arms for suspending the absorption tubes during weighings. Suitable balances have been put on the market by several manufacturers and of these Kuhlmann's "Schnellwaage, No. 4*a*"† is recommended. Its great advantage is constant sensitivity at varying loads. With this balance 20 mg. can be weighed with the same degree of accuracy as 2 mg. in the micro balance.

The technique of weighings is the same as that recommended by Pregl‡ such as acclimatisation, use of counterpoises, etc.

The following results of an analysis will illustrate the importance of the considerations mentioned above:

Substance.	Water.	Carbon dioxide.	Per cent. of	
			hydrogen.	carbon.
0.02458 g.	0.02037 g.	0.07219 g.	9.21	80.07
0.0246 g.	0.0204 g.	0.0722 g.	9.22	80.04
0.0245 g.	0.0204 g.	0.0722 g.	9.25	80.35
0.0246 g.	0.0203 g.	0.0721 g.	9.18	79.98

* *Cf.* Bobranski and Sucharda, *loc. cit.*, p. 28.

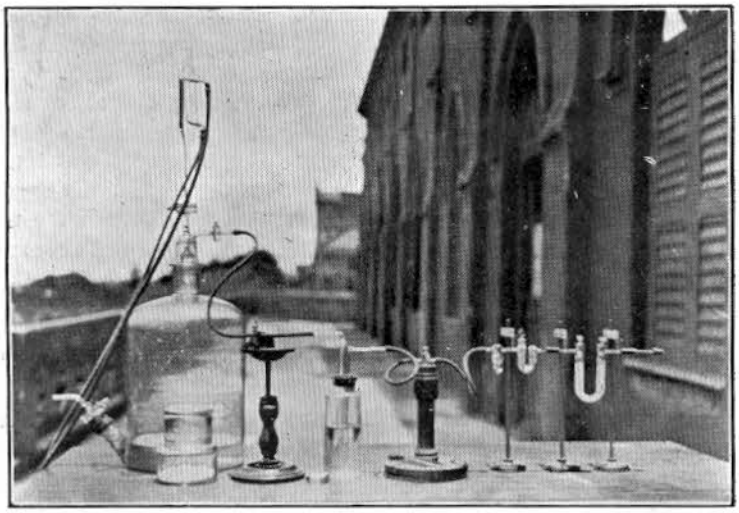
† Wilh. H. F. Kuhlmann, Steilshoperstrasse, 101-103, Hamburg 33.

‡ Pregl, "Quantitative Organic Mikroanalysis," 2nd ed., translated by Fyleman, 1930.

THE DETERMINATION OF CARBON AND HYDROGEN.

Outline.—20 to 30 mg. of material are taken for each analysis. The combustion tube is filled according to Pregl (*loc. cit.*, p. 30), and the oxidation is carried out in the presence of pure oxygen with platinised asbestos as catalyst. The products formed are received in suitably filled Blumer's absorption tubes and estimated.

Oxygen and its introduction into the combustion tube (Fig. 1). —Pure oxygen from liquid air,* obtained in steel cylinders, is bubbled through water and filled into the gas-holder. The connecting tubing is artificially aged according to Friedrich (Pregl, *loc. cit.*, p. 21). Similar tubing should also be employed for all connections between the gas-holder and the absorption train.



b a c d e f

Fig. 1.

The gas-holder is connected to Pregl's pressure regulator (*a*) with a precision pinch-cock (*b*) in between, to regulate the flow of oxygen. The pressure regulator is then connected to a U-tube with bubble counter (*d*) interposed by a stop-cock (*c*).

A few drops of pure 50 per cent. caustic potash are carefully run into the bubble counter, with the help of a thin tube,

* Oxygen generated from Oxone is also of the necessary purity.

so that the inner tube just touches the liquid. Small wads of cotton are inserted into the side tubes and the U-tube is nearly filled with ascarite and covered with a little cotton. The glass stopper is warmed, coated with Krönig's cement and screwed into position. The bubble counter is then calibrated as described by Pregl (*loc. cit.*, p. 27), "that is, the gas volume that passes the tube system in one minute must be correlated with the frequency of the bubbles."*

Next comes another U-tube (*e*) with ground stoppers. This is filled with dehydrite and covered with a little cotton. The stoppers are coated with good quality vacuum stop-cock grease and inserted into position. This U-tube is followed by a tapered thermometer tube (*f*) which leads into the combustion tube through a rubber cork. These absorption tubes (*d* and *e*) should be refilled after using them for 40 to 50 combustions.

The two U-tubes and second U-tube and thermometer tube are connected by means of 20 mm. lengths of thick-walled impregnated rubber tubing (Pregl, *loc. cit.*, p. 51)† and the glass parts should be flush with one another.

The combustion tube and its accessories (Figs. 2 and 2*a*).—The combustion tube is of hard Supremax glass of 12 mm. outer diameter and length, 53 cm.‡ To one end is joined a 2 cm. length of tubing of the same diameter as that of the side tubes of Blumer's absorption tubes.

The tube is filled as follows (Pregl, *loc. cit.*, p. 30):

It is repeatedly rinsed with chromic acid, washed with distilled water and carefully dried. Then a 7 mm. length of choking plug is inserted. This is accomplished by adding cleaned and freshly ignited asbestos in three portions through the wider end of the combustion tube and pressing it home

* This is accomplished thus: The U-tube is connected to the Mariotte flask (Pregl, *loc. cit.*, p. 49) and air is allowed to pass through. The displaced water is collected and measured. The number of bubbles corresponding to 10 c.c. are counted. From this result we get the number of bubbles corresponding to any given volume, say 4 or 5 c.c. This is checked by adjusting the pressure regulator and the syphon tube of the Mariotte flask, so that only 4 or 5 c.c. pass through per minute, and counting the number of bubbles. We thus derive the bubble frequency, namely, the number of bubbles per 10 or 15 seconds corresponding to a given volume.

† It is advisable to moisten the glass surfaces with a little glycerine and to wipe off all excess before inserting the rubber connections. Further the bore of rubber tubes also should be similarly treated, as recommended by Pregl, to prevent sticking.

‡ Sucharda and Bobranski, *loc. cit.*, p. 10.

each time with a clean glass rod. The degree of gaseous friction at this point is determined by inserting the tube into the hollow mortar maintained at 190°, and determining the number of c.c. of air that will pass through per minute, at a head of

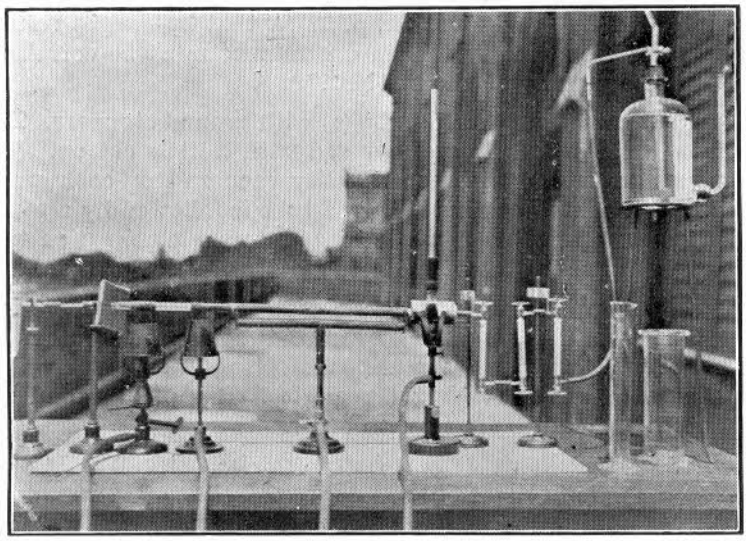
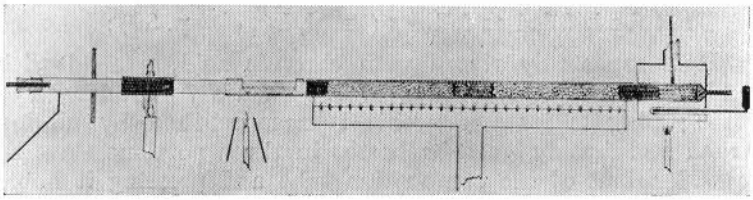


Fig. 2.



a_3 a_2 a_1

Fig. 2 a.

50 to 60 mm. of water in the pressure regulator. This should be 5 to 6 c.c. Otherwise adjustment is made accordingly either by pressing or loosening the plug.

Lead peroxide asbestos is inserted to a length of 30 mm. This is best accomplished by adding it in small portions and allowing it to slide along the tube. Tapping should be avoided as it will tend to compress the substance. A small wad of asbestos will keep it in position. At this stage it is advisable to

test the degree of gaseous friction inside the tube, as before, after inserting it into the heated mortar. Any diminution in bubble frequency will be due to close packing of lead peroxide asbestos. Adjustments are made so that the original bubble frequency corresponding to 5 or 6 c.c. is restored. The empty portion of the tube is cleaned by wiping thoroughly with a small piece of cloth attached to a long wire, in order to free it from particles of lead peroxide dust.

Then follows a layer of loosely packed silver wool or gauze* 30 mm. in length and again an asbestos wad.

The oxidising filling, consisting of a mixture of equal parts of wire form copper oxide and lead chromate (grains of 2 mm. diameter) is added to a length of 10 cm. and covered with an asbestos wad. The empty portion of the tube is wiped clean and a 30 mm. layer of 20 per cent. platinised asbestos is introduced. Care should be taken to allow the substance to slide along the wall of the tube as in the case of the introduction of lead peroxide asbestos. This is followed by another asbestos wad. It is advisable to test once again the degree of gaseous friction in the tube. For this purpose the tube is covered with the long brass cylinder (a_1) so that one end of it covers half the length of silver gauze, and is inserted into the heated mortar. The tube burner is lighted (*cf.* Sucharda and Bobranski, *loc. cit.*, p. 11), and after some time the bubble frequency is determined. If necessary, adjustments are made so that at least 4 to 5 c.c. of air pass through per minute.

The tube is again wiped and the filling completed by adding the oxidising mixture to a further length of 10 cm., then an asbestos wad and finally a roll of silver gauze 15 mm. in length. The empty portion of the tube is once again wiped clean.

The combustion tube is provided with an asbestos-lined thin-walled brass cylinder 26 cm. in length (a_1). This cylinder encloses the tube from the first silver gauze to about half of the second one, and is supported on a long burner.† The portion of the tube filled with lead peroxide asbestos is covered with asbestos paper and inserted into a mortar‡ whose temperature

* The silver used for tube filling must be cleaned by reducing it in a current of hydrogen and subsequently igniting it in a current of oxygen (Pregl, *loc. cit.*, p. 27).

† Sucharda and Bobranski, *loc. cit.*, p. 12. For an electrical combustion furnace, see Berger, *loc. cit.*

‡ A hollow mortar (Pregl, *loc. cit.*, p. 36) with boiling decaline is an advantage. However, when the mortar is used merely as an air bath, the temperature can be maintained surprisingly constant for long periods, by adjusting the micro-burner underneath.

can be maintained constant at about 190° by adjusting a micro-burner underneath.

The tube is also provided with two smaller asbestos-lined brass cylinders. The first of them is 60 mm. in length (a_2), and is placed next to the long cylinder. It is opened out at the top as indicated in the diagram and the combustion boat will be clearly visible from above. A micro-burner is placed underneath its wider end, for decomposing the material. The second small cylinder (a_3, 40 mm. in length) is placed at a distance of 35 to 40 mm. from (a_2), above a close-fitting freshly oxidised spiral of copper gauze introduced into the tube, and is heated by a Bunsen burner. An asbestos disc is put on the tube at a distance of 10 mm. from (a_3) to prevent the radiation of heat from affecting the rubber stopper. A further support to the tube is given near the mouth to prevent its bending as a result of continued heating during several combustions.

The absorption tubes.—Blumer's tubes (Sucharda and Bobranski, *loc. cit.*, p. 15) are employed.* For the absorption of water one of them is filled with dehydrite. The tube is first cleaned and dried. One of the stoppers is covered thinly with a small amount of good quality vacuum stop-cock grease and screwed into position. A small wad of cotton is inserted through the open end and pressed into position above the stopper with the help of a glass rod, so that it forms a thick layer of about 5 mm. The rest of the tube is filled with granular dehydrite, covered with a wad of cotton and the second stopper suitably greased is screwed home. It is advisable to make a mark on one of the stoppers so that only that end is always connected to the combustion tube.

The second Blumer's tube is filled in a similar manner so that $\frac{2}{3}$ its length is occupied by ascarite and $\frac{1}{3}$ by dehydrite, with a 2 mm. layer of cotton separating the two.

The tubes are joined to one another by a rubber connection and all the air is displaced by passing through them slowly about 150 c.c. of oxygen. The stoppers are then turned through 180°.

Before weighing, the side tubes of each of them are cleaned by means of a small wad of cotton attached to a steel wire. They are successively wiped with moist flannel and 2 pairs of clean chamois leather pieces,† and allowed to stand on a frame

* The Pregl tubes are unsuitable because of the formation of relatively larger amounts of water which tends to choke the capillary constructions thereby causing back pressure.

† Sucharda and Bobranski, *loc. cit.*, p. 16.

(Pregl, *loc. cit.*, p. 44) for ten minutes. They are then suspended in the balance and their weights determined. An aluminium fork is employed to handle them after wiping.

The dehydrite tube usually lasts for 30 to 40 combustions and the ascarite tube for about 4 to 6. It is advisable to work with two pairs when carrying out a number of successive combustions and to keep a good stock of spare tubes.*

Preliminary roasting and blank tests.—Preliminary roasting is carried out thus: The long burner and the micro-burner under the heating mortar are lighted. The temperature in the mortar is adjusted to 190°. The narrow end of the combustion tube is connected to the Mariotte flask and the syphon tube is so adjusted that 5 c.c. of air per minute are drawn through the combustion tube. The brass cylinder (a_3) is moved over to the end of the combustion tube and heated strongly by a Bunsen burner. The flame, with the brass cylinder above, is gradually moved towards the tube filling. The empty portion of the tube is thus thoroughly ignited and the burner removed. When the open end of the combustion tube is sufficiently cool, the rubber stopper is inserted,† and the Mariotte flask is disconnected. The absorption train is then joined on by means of the thermometer capillary and oxygen is allowed to pass through at the rate of 5 c.c. per minute by adjusting the pressure regulator.‡ The roasting is continued for at least 3 hours. The apparatus is then ready for carrying out a blank test.

With the long burner lighted and the mortar maintained at 190°, the absorption tubes are weighed as described already. The ascarite tube is connected with the dehydrite tube in such a manner that the gases coming from the latter pass through ascarite first. A piece of impregnated rubber tubing 20 mm. in length is used to join the two. With another piece of the same

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* The entire apparatus can be obtained from the firm, Greiner and Friedrichs, G.m.b.H., Stützerbach in Thür. Mention may also be made of Paul Haack, Garelligasse 4, Vienna IX/3, for some specialities like the hollow mortar, precision pinch-cock, Krönig's cement, rubber tubing, etc.

Oxone, ascarite and dehydrite are obtained from the Central Scientific Co., 460, East Ohio Street, Chicago, and the other chemicals "nach Pregl" from E. Merck, Darmstadt.

† The bore and the outer surface of the rubber stopper should be moistened with just a trace of glycerine and wiped clean. This should be done from time to time in order to prevent its sticking to the glass surface.

‡ After adjusting the pressure regulator now, the pressure under which oxygen enters the tube should not be altered. Occasionally, however, it may become necessary to determine the bubble frequency when the narrow end of the tube is open and to make minor adjustments.

length, the other end of the ascarite tube is connected to a Blumer's tube filled with dehydrite. This acts as a safety tube. The weighed dehydrite tube is then attached to the combustion tube by another piece of impregnated tube (15 mm. length).

The bores of the connecting pieces of impregnated tubes should be moistened with glycerine each time before use, by passing through them a small wad of cotton containing a little glycerine. Any excess is wiped out by means of a dry cotton wad. The glass parts in all the above connections should be flush with one another.

The safety tube is connected to the Mariotte flask and the stoppers of all the Blumer's tubes are so turned that oxygen passes through the combustion tube. The bubble frequency, corresponding to 5 c.c. per minute, is adjusted by raising or lowering the syphon tube of the Mariotte flask.

A few small asbestos paper discs are put between the mortar and the first rubber connection to prevent its rapid deterioration on account of radiated heat. The copper heater from the mortar is placed on the open portion of the side arm of the dehydrite tube.

The roasting is continued for one hour. Occasional attention is paid to maintain the constancy of temperature in the mortar. At the end of the period, the Mariotte flask is disconnected. The absorption tubes are closed and detached. The safety tube is connected to the neck of the combustion tube by means of impregnated tubing.

The absorption tubes are cleaned, wiped and after remaining for ten minutes on the stand, are weighed. If the apparatus is in a satisfactory condition, they should show no increase in weight in the fourth place after the decimal; otherwise the entire apparatus is examined carefully for leaks, etc., and the roasting continued till constancy of weight in blank tests is ensured.

When the apparatus is to be set aside for the day, the burners are all put out. The tube is allowed to cool and oxygen from the gas-holder is stopped. The stop-cock (*c*) and the stoppers of the U-tube (*e*) are turned through 90° and finally that at the end of the safety tube through 180°.

A tube thus prepared lasts for at least 75 to 100 combustions. If a number of halogenated organic compounds are

being analysed, it is necessary to clean the smaller silver gauze, occasionally, as described already.

The course of analysis.—Before the analysis of unknown substances is undertaken, it is advisable to carry out the combustion of one or two known substances, such as acetanilide, allantoin, etc.

In carrying out a combustion, it is best to follow closely the following procedure:

1. The stoppers of the stop-cock (*c*), the U-tube (*e*) and of the terminal safety tube are turned to allow the passage of oxygen through the combustion tube. Oxygen is let in from the gas holder and its flow into the pressure regulator is controlled by the precision pinch-cock. The long burner and the micro-burner under the mortar are lighted and the temperature of the mortar is regulated to 190°.

2. The absorption tubes are cleaned, wiped and placed on the stand.

3. Oxygen is stopped from the gas holder and the empty portion of the tube is rapidly ignited in the manner already described. The absorption train is connected again and oxygen is let in. The bubble frequency is tested. The impregnated rubber connections are treated with glycerine and wiped. The spiral of copper gauze is ignited and allowed to cool in a desiccator.

4. The substance (20 to 30 mg.) is weighed into a suitable platinum or porcelain boat* and placed in a desiccator. The absorption tubes are then weighed, inter-connected and, together with the material, are moved near to the combustion train.

5. The terminal safety tube is disconnected and the tip of the combustion tube is carefully wiped. The dehydrite tube is attached to the combustion tube and the ascarite tube to the safety tube, which in turn is connected to the Mariotte flask with its syphon tube raised. Asbestos discs are placed between the mortar and the first rubber connection, and the copper heater on the open portion of the side arm of the dehydrite tube. The stop-cock of the gas holder is closed.

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* Before use the boats should be boiled with nitric acid, washed, dried, ignited and allowed to cool on copper block inside a desiccator.

For the manipulation of liquids with low boiling points, see Pregl, *loc. cit.*, p. 70 and Sucharda and Bobranski, *loc. cit.*, p. 27. In the case of high boiling liquids (b. p. above 200°) one drop is put into the boat and weighed out in the usual manner.

6. The rubber cork is removed from the mouth of the combustion tube, the boat is inserted by means of a pair of forceps and pushed in with the help of a clean dry glass rod, so that it becomes visible from the open portion of the brass cylinder (a_2) and is not more than 1 cm. from the tube filling. The oxidised copper spiral is introduced into position ; the rubber stopper is then inserted and the combustion tube is connected with the absorption train. The stoppers of the Blumer's tube are all suitably turned to allow the passage of gas.

The apparatus is tested for any leaks by closing the stop-cock (c) and lowering the syphon tube of the Mariotte flask. After a few seconds no more bubbles should rise in the flask.

7. Oxygen is let in and bubble frequency is adjusted by raising or lowering the syphon tube of the Mariotte flask.

8. The burner under the brass cylinder (a_3) is lighted. When it is red hot, a micro-burner with a small flame, the size of which depends upon the nature of the substance that is being oxidised, is placed under the wider end of the brass cylinder (a_2).

After a little time the substance begins to decompose. This should be slow and gradual and it is best to work with a very low flame. At this stage it is usual to notice a temporary diminution in bubble frequency. The entry of oxygen, however, should not stop and any back pressure, as will be indicated in the bubble counter, should be carefully avoided.

The copper heater is used to drive any moisture condensing in the side arm of the dehydrite tube into its stopper. Occasional attention is also paid to maintain constancy of temperature at 190° in the mortar.

The decomposition will be over in a short time (2 to 5 minutes). The micro-burner is replaced by a good Bunsen burner and the charred particles are completely burnt out.

At this stage, the passage of bubbles in the Mariotte flask will become very slow although the bubble counter may indicate a slightly increased rate of entry of oxygen into the combustion tube. Water will collect in the stopper of the dehydrite tube and the absorption of carbon dioxide in the ascarite tube will be apparent.

9. The regular combustion is now over. 100 to 150 c.c. of oxygen are allowed to pass through, in order to wash out all the products of combustion into the absorption tubes. This

volume is determined by measuring the water issuing from the syphon tube in a graduated cylinder.

During this period the Bunsen burner, together with the brass cylinder (a_3), is gradually moved till the latter joins the cylinder (a_2). In this manner the empty portion of the tube is thoroughly ignited.

If successive combustions are to be carried out, material is weighed out into a second boat and another set of Blumer's tubes are got ready.

10. As soon as the required volume of water is collected in the measuring cylinder, the syphon tube is raised, and the absorption tubes are disconnected after closing the stoppers.

If a second combustion is to be carried out, another set of weighed absorption tubes are attached to the combustion tube, and the procedure repeated. On opening the rubber cork, the copper spiral and the boat are removed by means of a platinum hook. The second boat followed by the copper spiral is introduced, and the cork is re-inserted. Otherwise, the tube is allowed to stand by in the manner already described.

The absorption tubes are cleaned, wiped and weighed in the usual manner and the results are then calculated.

A variety of substances with varying chemical compositions have been analysed and the accuracy of the determinations is ± 0.2 per cent.

Some results:

Substance and its formula.	Wt. taken.	H_2O	CO_2	Per cent. of			
				Hydrogen		Carbon	
				Found	Theoretical	Found	Theoretical
	g.	g.	g.				
p-Phenyl phenacyl ester of lignoceric acid, $C_{38}H_{58}O_3$.	0.02408	0.02250	0.07164	10.4	10.4	81.1	81.1
Allantoin, $C_4H_6N_4O_3$.	0.01361	0.00477	0.01507	3.9	3.8	30.2	30.4
2:4 Dihydroxy isophthalic acid, $C_8H_6O_6$.	0.02982	0.00853	0.05304	3.2	3.1	48.5	48.5
Diethyl ester of 2:4 dihydroxy isophthalic acid, $C_{12}H_{14}O_6$.	0.02766	0.01354	0.05742	5.4	5.5	56.6	56.7
p-Bromphenacyl ester of $C_{23}H_{46}O_2$, $C_{31}H_{51}O_3Br$.	0.01664	0.01375	0.04120	9.2	9.3	67.5	67.5

THE DETERMINATION OF NITROGEN.

Although the volumetric determination of nitrogen in organic compounds, according to the micro method of Pregl, yields accurate results without considerable difficulty, still, where a micro balance is not available, the semi-micro method affords a valuable alternative. Suitable methods and apparatus have been advanced by Berl and Burckhardt (*Ber.*, 1926, **59**, 897), Lauer and Sunde (*Mikrochemie*, Preglfestschrift, 1929, p. 239) and Sucharda and Bobranski (*loc. cit.*, p. 30). The following procedure which is a close application of Pregl's micro method to centigramme analysis has been found to be equally accurate.

The carbon dioxide generator.—Medium sized pieces of marble are kept in water and boiled for a few hours under diminished pressure. They are then washed with a little dilute hydrochloric acid and charged into the middle bulb of a fairly large-sized Kipp's apparatus. The delivery tube (*a*) is provided with a stop-cock (c_1) and is so arranged that the gas is drawn from the top portion of the chamber. A mixture of equal parts of concentrated hydrochloric acid and boiled water is added through the upper bulb. The stop-cock on the delivery tube is opened for a short time so that there is vigorous evolution of carbon dioxide. This is repeated several times in order to drive out all the air in the apparatus. Also, one or two larger pieces of marble are inserted into the central tube to complete the de-æration. A Kipp charged in this manner is allowed to stand for some days during which the process of de-æration is repeated a few times.* It will then deliver carbon dioxide of the specifications of Pregl as judged by the formation of micro-bubbles when slowly let into the azotometer.

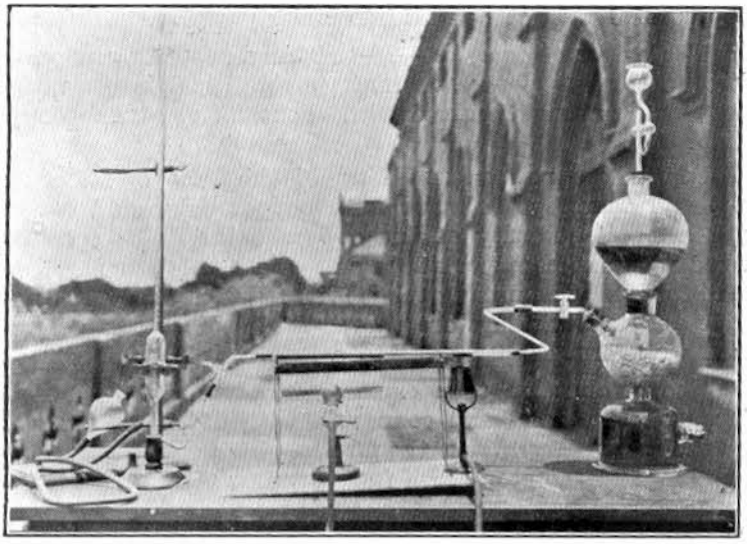
The combustion tube, its accessories and the azotometer (Fig. 3).—The delivery tube of Kipp's apparatus is connected by means of pressure tubing to a *Z*-form capillary tube which leads into the combustion tube through a rubber cork.

The combustion tube is similar to the one employed for the determination of carbon and hydrogen but of a total length of 45 cm. For filling it, it is first cleaned and dried. A small asbestos wad inserted through the wider end is pushed to the neck. A layer of freshly ignited wire form copper oxide 90 mm. in length is introduced and covered with a small

* The de-æration process is repeated once a week since hydrochloric acid in the upper bulb comes into contact with air after some time, in spite of the safety tube.

amount of asbestos. This is followed by a 50 mm. length of freshly reduced copper wire* and covered with a little asbestos. The materials added till now form the permanent filling of the tube.

The combustion tube is provided with two asbestos-lined brass cylinders, one 19 cm., and the other 35 mm. in length. The former (b_1) is placed in such a manner that one end of it is about



c_2 b_1 b_2 c_1 a

Fig. 3.

8 mm. from the neck of the tube. The smaller one (b_2) is used to cover the tube when the substance is being heated by a Bunsen burner. The combustion tube is supported on a stand and is heated along the length covered by the longer cylinder by means of a tube burner.

The narrow end of the combustion tube is connected to Pregl's stop-cock with long handle† (c_2) which in turn is joined to an azotometer similar to the one employed by Berl and Burckhardt (*loc. cit.*), but with only an ordinary stop-cock above the graduated end. The Pregl's stop-cock is introduced

* See Pregl, *loc. cit.*, p. 93. This should be replaced after 15 to 20 combustions.

† This stop-cock should be occasionally cleaned in order to remove condensed water.

to regulate the rate of entry of gas bubbles into the azotometer. The azotometer is filled with mercury up to a level just above the lower side tube and 50 per cent. potassium hydroxide, prepared according to Pregl (*loc. cit.*, p. 99), is introduced into the levelling bulb.*

When a tube is filled for the first time, it is roasted for 10 to 15 minutes in a current of carbon dioxide. Stop-cock (c_2) is then closed and that on the delivery tube opened so that the tube is filled with carbon dioxide under pressure.

The course of analysis.—20 to 30 mg. depending upon the nitrogen content of the substance, are weighed into a mixing tube† and thoroughly mixed with about 3 c.c. of fine copper oxide.

Above the permanent filling of the tube is introduced another 10 cm. layer of wire form copper oxide and covered with a small quantity of fine copper oxide. The substance mixed with fine copper oxide is added into the tube through a small funnel. The mixing tube is rinsed twice with fine copper oxide and the washings are poured into the combustion tube. Then wire form copper oxide to a length of 8 cm. is added.‡

The combustion tube enclosed in the brass cylinders is placed on the stand and connected with the Kipp generator and the azotometer. With the levelling bulb of the azotometer kept low and the stop-cocks fully open, a vigorous stream of carbon dioxide is passed through the tube for 4 to 5 minutes. The long burner is then lighted, the stop-cock (c_2) is closed and the azotometer filled with caustic potash by raising the levelling tube. By means of the long handle of the stop-cock (c_2) carbon dioxide is allowed to enter into the azotometer at the rate of a bubble per second. The bubbles should be completely absorbed leaving only the micro-bubbles to rise in the stem. Otherwise the process of washing out is continued as before and the apparatus is tested to make certain that all the air has been removed.

With the azotometer set, the stop-cock (c_1) is closed and (c_2) is fully opened. The combustion is commenced at the farther end of the tube filling, with a Bunsen burner keeping the brass cylinder (b_2) above. The flame is moved gradually towards the

* The complete apparatus can be obtained from the firm Greiner and Friedrichs.

† An ordinary test tube 50 mm. long and 12 mm. in diameter is used. A tight-fitting cork without any grains is used to close the mouth when shaking.

‡ The filling after the reduced copper is removed each time a combustion is to be carried out and sieved. The two varieties of copper oxide are ignited separately and used for subsequent tube fillings.

long burner so that the substance is very slowly oxidised as indicated by the entry of gas into the azotometer, which should at no time contain more than two bubbles at the same time. The combustion is completed when the Bunsen burner is brought close to the long burner.

Then commences the washing out process. The stop-cock (c_2) is closed and (c_1) is fully opened. The former is very gently adjusted so that gas begins to enter the azotometer at the rate of a bubble per second. The washing is continued till micro-bubbles once again begin to ascend in the tube. During this period the portion of the tube outside the long burner is ignited strongly and the burners are put out.

A few minutes after the micro-bubbles begin to ascend in the tube, the stop-cock (c_2) and the stop-cock on the azotometer side tube are completely closed, and the azotometer is disconnected and moved to a cooler place. The levelling bulb is kept raised and a thermometer is kept near the gas in the stem. After 15 minutes, the volume is read accurately with the help of a lens, after equalizing the pressures by means of the levelling bulb. The temperature and the barometric pressure are noted.

From the volume thus read 1.2 per cent.* is deducted to make allowance for the volume of the alkali adhering to the sides and the results calculated in the usual manner. The accuracy of estimations according to this method is $+0.2$ per cent. and a determination usually takes $1\frac{1}{2}$ to 2 hours.

Some results :

Substance and its formula.	Wt. taken.	Volume of Nitrogen.	t°.	Pressure in mm. of mercury.	Per cent. of Nitrogen		
					Calculated from uncorrected volume	Calculated from corrected volume.	Theoretical.
Acetanilide, .. C_8H_9NO.	0.0214 g.	2.23 c.c.	25°	683	10.7	10.6	10.4
Naphthylamine, .. $C_{10}H_9N$.	0.0233 g.	2.25 c.c.	26°	683	9.9	9.8	9.8
Salicylamide, .. $C_7H_7O_2N$.	0.0217 g.	2.20 c.c.	24°	684	10.5	10.4	10.2
m-Nitro phenol, .. $C_6H_5O_3N$.	0.0237 g.	2.40 c.c.	25°	684	10.4	10.3	10.1
Allantoin, .. $C_4H_6N_4O_3$.	0.0117 g.	3.86 c.c.	25°	683	35.9	35.5	35.5

* *Cf.* Sucharda and Bobranski, *loc. cit.*, p. 34. Weygand (*loc. cit.*, p. 60) considers that the above correction is arbitrary, although its use with several known substances has given accurate results. However, the azotometer may also be calibrated according to the method of Berl and Burckhardt (*loc. cit.*).